

Symposium lectures abstracts

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Tuberculous infection – continuous challenge

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What medical biochemist should know about tuberculosis infection?

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Immune response to *M. tuberculosis* is a classic example of the cell-mediated immunity. After uptake of *M. tuberculosis* in alveolar macrophages a several scenarios are possible: in healthy subjects *M. tuberculosis* may be destroyed immediately (in this case no adaptive T-cell response is developed). Mycobacteria which escape this destruction will multiply and macrophages will be disrupted. Inflammatory cells, including blood monocytes are attracted to the lung. Monocytes will differentiate into macrophages and ingest (not destroy) mycobacteria, so mycobacteria may multiply logarithmically. Within 2 to 6 weeks, T-cell-mediated immunity develops, i.e. antigen-specific T-lymphocytes arrive, proliferate, and activate macrophages (resulting in granuloma formation) to kill the intracellular mycobacteria. Subsequently, central necrosis inhibits extracellular growth of *M. tuberculosis*. In 90% of people infection may become dormant, and 10% of people may develop tuberculosis (TB). Protective anti-mycobacterial immunity involves many T-lymphocytes activating the macrophages and their microbicidal functions. Their activation requires the release of numerous cytokines, such as IL-12, IL-10, interferon-gamma (IFN- γ) or tumour necrosis factor alpha (TNF- α).

Young children and elderly persons have the highest risk of development TB, since they have relatively weak immune defences, because of immature system (children) or age-related immune dysfunction (elderly people). Persons with impaired immunity due to HIV infection or patients who must receive anti-TNF- α medication are at high risk of TB development.

Microbiologic techniques (eg. finding of *M. tuberculosis* in culture) enable detection *M. tuberculosis* in biological specimens, and biochemical methods (eg. ex vivo determination of IFN- γ) enable detection tuberculosis infection even in latent stage.

Tuberculosis and anti-cytokine therapy

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Patients with rheumatoid arthritis, RA, have greater rates of infections comparing with general population. Most common infections are respiratory tract infections and also skin and soft tissue infections. Treatment guidelines imply decisive and aggressive immunosuppressive treatment early in the course of the disease, just after the diagnosis of RA is established. The introduction of biological drugs has increased the risk of tuberculosis, especially in the case with the use of drugs that inhibit tumor necrosis factor alpha, TNF- α .

TNF- α , especially transmembrane TNF- α , plays an important role in innate immunity and immune defense focused on *Mycobacterium tuberculosis*. The mechanisms that are inhibited by using anti-TNF- α drugs are important for maintaining the latent phase of tuberculosis (LTBI - latent tuberculosis infection). The key role of TNF- α in the defense against tuberculosis has been showed in the experiments with knock-out mice which showed that the cytokine TNF- α is necessary for granuloma formation and its maintenance, and that it have equally important role in the macrophages activation. Excessive inhibition of TNF- α leads to exacerbation of TB.

The importance of careful screening of patients for tuberculosis before the introduction of biological drugs in to the treatment of inflammatory rheumatic diseases was also recognized by the Croatian Society for Rheumatology and the Croatian Respiratory Society, so the joint guidelines for the diagnosis of latent tuberculosis in vaccinated patients before the introduction of anti-TNF- α therapy were adopted. Early introduction and application of these guidelines results in a low occurrence of tuberculosis in Croatian patients on biological therapies.

Tuberculosis in HIV infected patients

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Because of the high burden of disease in developed countries, *Mycobacterium tuberculosis* infection is the most common opportunistic disease in HIV infected patients. In Croatia, tuberculosis is the second most common opportunistic infection diagnosed in HIV infected individuals. Despite recent advances, the diagnosis of tuberculosis is still challenging and empiric antituberculosis therapy is still necessary, particularly in resource limited settings.

A diagnostic algorithm including assessment of symptoms (fever, cough, and night sweats), chest radiography, sputum smear examination and CD4 cell counts determination can be used to reliably exclude the diagnosis of tuberculosis in HIV infected patients. In smear negative specimens new diagnostic tools such as Xpert MTB/RIF can improve the diagnostic yield. In the early stages of HIV-infection the clinical symptoms of tuberculosis are similar to those in HIV-negative patients. Extrapulmonary tuberculosis occurs predominantly in patients with CD4+ cells below 200/mm³, most commonly affecting the cervical lymph nodes. Other extrapulmonary manifestations include tuberculosis meningitis, miliary or disseminated tuberculosis, however, any organ system can be involved.

Simultaneous antiretroviral and antituberculosis therapy is challenging because of the associated mortality of tuberculosis in severe immunodeficiency, the so called immune reconstruction syndrome and drug interactions. Treatment of tuberculosis has a priority. However, several studies found that the earlier antiretroviral treatment in patients with tuberculosis, particularly in patients with less than 50 CD4+ cells/mm³ or less than 200 CD4+ cells is associated with less mortality. Hence it is recommended to start antiretroviral therapy within two-weeks of antituberculosis therapy in patients with 50-100 CD4+ cells/mm³.

Laboratory diagnosis of latent tuberculosis infection

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Laboratory diagnosis of latent tuberculosis infection (LTBI) is based on the measurement of the host immune response against *Mycobacterium (M.) tuberculosis*. The blood test determining interferon-gamma (IFN- γ) released from effector T-lymphocytes (interferon-gamma release assay, IGRA) upon stimulation with *M. tuberculosis* specific peptides.

The differences between two commercially available IGRAs are the type of sample (whole peripheral blood or purified peripheral blood mononuclear cells), the number of specific antigens for ex vivo stimulation and the method for detection of IFN- γ production (the enzyme-linked immunosorbent assay determines IFN- γ concentration and the enzyme-linked immunosorbent spot determines the percentage IFN- γ releasing lymphocyte).

The high specificity (about 99%) and sensitivity (65-95%) of IGRAs supports the use of IGRAs in the diagnosis of LTBI in risk individuals but predictive values of IGRAs to predict risk for reactivation of LTBI to active tuberculosis are not known at this moment.

The advantage of IGRAs is the possibility of performance quality control. Positive control is used to assess the preanalytical factors as patient's immune status and sample manipulation while negative control offers an insight into the nonspecific IFN- γ level present in the circulation or indicates the presence of interference antibodies in the sample. The unsatisfactory result for control sample is classified as the uninterpretable result of IGRAs. The disadvantage of IGRAs is their inability to distinguish between latent and active tuberculosis infection.

The introduction of IGRAs to routine clinical practice has improved the diagnosis of LTBI but each national tuberculosis control program should evaluate IGRAs for their own occasion.

Indeterminate results of ex vivo Interferon-gamma determination in non-immunosuppressed children

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Immunosuppressed individuals due to pathologic conditions (eg. HIV infection, hematologic malignancies, chronic renal failure) or therapeutic procedures (eg. organ transplantation, chemotherapy, immunosuppressive therapy with tumor necrosis factor α (TNF- α), therapy with systemic corticosteroids) are at high risk for reactivation of latent tuberculosis infection (LTBI), and require timely identification.

Laboratory diagnosis of LTBI is based on the blood test determining interferon-gamma (IFN- γ) released from effector T-lymphocytes (interferon-gamma release assay, IGRA) upon stimulation with *M. tuberculosis* specific peptides.

Two children with indeterminate results of IGRA are presented. They were free from congenital or acquired immunodeficiency disorders, as shown by their history data, clinical examination and laboratory findings.

Case 1: a 15-month-old male infant was referred for testing because of cough and febrile condition persisting for a week before admission. Status: febrile, eupneic, pale, tachycardia, serous nasal discharge, pharyngeal congestion, normal breath sounds over lungs on auscultation. Examination and test findings indicated the diagnosis of pneumonia.

Case 2: a 5.5-year-old female child was referred for testing due to persistent cough and subfebrile condition with serous nasal discharge for three days before admission. She was positive for contact with tuberculosis patient, as her uncle has been treated for microbiologically positive lung tuberculosis 3 years before. Examination and test findings indicated the diagnosis of lung tuberculosis.

As no indeterminate results were found on repeat IGRA, it was assumed that acute bacterial inflam-

mation (acute pneumonia or tuberculosis), could produce the indeterminate results recorded on initial testing. In acute inflammation IGRA should be delayed.

Ex vivo determination of IFN- γ in health care workers

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Detection and follow-up the blood test determining interferon-gamma (IFN- γ) released from effector T-lymphocytes (interferon-gamma release assay, IGRA) upon stimulation with *M. tuberculosis* specific peptides should be performed in subjects at an increased risk of *M. tuberculosis* infection such as health care workers (HCW) in chest hospital and immunocompromised subjects.

Two cases of HCW with type 1 diabetes mellitus (T1DM) and positive anti-thyroglobulin (anti-Tg) autoantibodies, respectively, are presented.

Case 1: a 46 year-old female HCW who has been having T1DM for 20 years underwent routine screening for Latent tuberculosis infection (LTBI). She was not acutely ill, she had normal body temperature, was eupneic and lung auscultation show normal breathing. Tuberculin skin test (TST) was negative and IGRA was positive (4.7 kIU/L). Positive IGRA indicated the LTBI, while TST result was false negative. Antituberculosis prophylaxis was not used.

Case 2: a 57 year-old clinically healthy female was included in an annual screening for LTBI. Initial testing showed positive TST and IGRA (5.2 kIU/L) and elevated levels of anti-Tg (86.4 kIU/L). She was free from acute disease, afebrile, eupneic, with normal breath sounds on auscultation. One year later IGRA was still positive. Antituberculosis prophylaxis was not used.

Although isoniazid prophylaxis was not used in these subjects, tuberculosis did not develop during the 4-year follow-up period.